European Journal of Plant Pathology **109:** 341–349, 2003.

Integrated control of apple postharvest pathogens and survival of biocontrol yeasts in semi-commercial conditions

Giuseppe Lima, Filippo De Curtis, Raffaello Castoria and Vincenzo De Cicco
Department of Animal, Plant and Environmental Sciences, University of Molise, Via F. De Sanctis, 86100 Campobasso, Italy (Phone: +39 874 404698; Fax: +39 874 404678; E-mail: lima@unimol.it)

Accepted 9 December 2002

Key words: biological control, fungal decays, fungicides, yeast fingerprints

Abstract

The biocontrol yeast isolates *Rhodotorula glutinis* LS11, *Cryptococcus laurentii* LS28 and *Aureobasidium pullulans* LS30 were tested against *Botrytis cinerea* and *Penicillium expansum* on apples artificially inoculated and stored at 3 and 20 °C. Isolates LS28 and LS30 were most effective, consistently resulting in high reductions of fungal decay, while isolate LS11 was effective only on apples stored at 3 °C. The yeasts showed good *in vitro* resistance to dicarboximides and copper fungicides, while they were inhibited by triazoles. Isolate LS11, in contrast to LS28 and LS30, was also inhibited by benzimidazoles. The yeasts were tested on naturally-infected apples in semi-commercial conditions for 2 years. They were applied twice: soon after harvesting and 20 days later, at the beginning of the cold storage. The antagonists significantly reduced fungal decay when combined with a low dosage of benomyl showing an activity comparable to that exerted by the fungicide alone at full dosage. Periodical monitoring of the epiphytic biocontrol yeast populations in both the field and cold room showed a good rate of survival of the antagonists on the skin of treated apples. Specific fingerprints relying on amplified restriction length polymorphism (AFLP) were used to integrate the morphology-based monitoring of the yeasts.

Introduction

Postharvest fungal pathogens cause severe losses on apples during postharvest storage and commercialization. The most aggressive pathogens are *Penicillium expansum*, *Botrytis cinerea*, *Monilinia* spp. and *Phlyctaena vagabunda* (= *Gloeosporium album*). Pre- and postharvest applications of synthetic fungicides are frequently used to control postharvest rots. However, these products involve risks for consumers' health (Caffarelli et al., 1999) and for onset of pathogen-resistant strains (Spotts and Cervantes, 1986; Guizzardi et al., 1995; Stehmann and De Ward, 1996). Therefore, new strategies have been proposed to reduce or replace chemicals to control postharvest diseases (Wilson and Wisniewski, 1994; El Ghaouth, 1997). In particular, the use of antagonistic microorganisms seems to be effective in reducing the incidence of postharvest fungal pathogen of different fruits, both in small-scale experiments and in semi-commercial conditions (Wilson and Wisniewski, 1994; Jijakli et al., 1999; Lima et al., 1999). At present an increasing interest is being devoted to yeasts isolated from fruit surfaces because of their ability to survive under different conditions and the absence of toxic effects to laboratory experimental animals (Arras et al., 1999; Usall et al., 2001). Yeast-based biofungicides are already commercially available in the USA, Israel (Aspire®, based on *Candida oleophila*) and South Africa (Yield Plus®, based on *Cryptococcus albidus*) for the control of postharvest rots of pome and/or citrus fruits (Droby et al., 2002). In order to further encourage companies, particularly in Europe, to develop new and highly effective biofungicides based on biocontrol yeasts it is necessary to make their activity more reliable by combining them with additives or other control strategies, including low dosages of fungicides, in integrated control programmes

(Chand-Goyal and Spotts, 1997; Ippolito et al., 1998; Droby et al., 2002). The antagonist operates mainly through an efficient competition essentially based on a preventive colonization of fruit wounds (Castoria et al., 1997; 2001). Therefore, application of biocontrol agents in the field has been suggested (Lima et al., 1997; Ippolito and Nigro, 2000). In these conditions, specific molecular markers are necessary for both unequivocal identification and monitoring of released antagonists (Nigro et al., 1999; Schena et al., 2001).

In this paper, we report the results of a 2 year investigation aimed at assessing the efficacy of an integrated control method of postharvest rots of apples which combines the application of biocontrol yeast isolates with a low dosage of fungicide. The antagonists, selected and characterized for their high activity against postharvest rots on several fruits, were assayed alone in small-scale experiments and in combination with a reduced rate of benomyl in commercial apple groves. Specific fingerprints of the antagonists were developed by amplified fragment length polymorphism (AFLP) technique and were utilized to integrate the monitoring of biocontrol yeasts in semi-commercial conditions.

Materials and methods

Antagonists and pathogens

The yeasts *Rhodotorula glutinis* (LS11), isolated from olives, *Cryptococcus laurentii* (LS28), isolated from apples, and the yeast-like fungus *Aureobasidium pullulans* (LS30), isolated from apples, were the biocontrol agents. The fungal pathogens *B. cinerea* and *P. expansum* were isolated from decayed fruits. In order to obtain the conidial suspensions for fruit inoculation, each pathogen was grown on potato dextrose agar (PDA) under fluorescent light for 5–7 days at 21 °C. Five millilitres of sterile distilled water containing 0.05% Tween 20 were poured in each plate, conidia were scraped from the agar by a sterile loop and suspension was filtered through 4 layers of cheesecloth. Concentrations were adjusted to 1×10^5 and 2×10^4 conidia ml^{-1} for *B. cinerea* and *P. expansum*, respectively.

Small-scale experiments on wounded apples

The experiments were performed on apples cv. Annurca harvested from orchards located in Southern Italy. Fruits were surface disinfected by immersion for 1–2 min in a sodium hypochlorite solution (2% active chlorine), rinsed twice with sterile distilled water and dried at room temperature. Four wounds (3 mm wide × 3 mm deep) were produced around the pedicel zone on each fruit. Thirty microlitres of antagonist cell suspension (10^7 cfu ml^{-1}) were placed in each wound, while the same volume of sterile distilled water was placed in control wounds. After 2 h at room temperature each wound was inoculated with 15 µl of spore suspensions of *B. cinerea* or *P. expansum*. Fruits were incubated in the dark at 20 °C for 5 days or at 3 °C for 30 days with 95–98% RH. Decay, expressed as percentages of wounds showing rot symptoms, was periodically assessed and the experiments were stopped when 90–100% wounds were rotted in control fruits. Each treatment included 5 replications and each replication consisted of 6 fruits. The experiments were performed twice and data were pooled and processed by statistical analysis.

Sensitivity of antagonists to fungicides

The biocontrol yeasts LS11, LS28 and LS30 were tested *in vitro* for their sensitivity to the following fungicides: Benlate (active ingredient (a.i.): benomyl, 50% – Manufacturer (Man): DuPont), Siatek 42F (a.i.: thiabendazole, 41.8% – Man: Siapa), Azuram (a.i.: copper oxychloride, 40% – Man: DuPont), Topas 10EC (a.i.: penconazole, 10.2% – Man: Syngenta), Folicur WG (a.i.: tebuconazole, 25% – Man: Bayer), Sumisclex (a.i.: procymidone, 50% – Man: Sumimoto) and Ronilan FL (a.i.: vinclozolin, 41.3% – Man: Basf). The assays were performed on basal yeast agar (BYA: 10 g bacteriological peptone, 1 g yeast extract, 20 g dextrose, 18 g agar, 1 l distilled water). Each fungicide was suspended in distilled water and mixed with the medium at 45 °C to obtain final concentrations of 0, 10, 20, 50, 100 and 500 mg l^{-1} of trade product (Table 1). Each plate (4 replications per treatment) was poured with 100 µl of yeast suspension containing about 100 cells and incubated for 7 days at 23 °C. In each plate the growing yeast colonies were counted and minimum inhibitory concentration (MIC), which represents the lowest concentration totally inhibiting the development of colonies from yeast cells, was assessed for each biocontrol agent and fungicide.

Semi-commercial apple treatments

The experiments were carried out in commercial groves of Campania (Region of Southern Italy) in the years

Table 1. Minimum inhibitory concentration (mg l^{-1}) of some trade-products of common fungicides towards the *in vitro* growth of the biocontrol yeasts *R. glutinis* (LS11), *C. laurentii* (LS28) and *A. pullulans* (LS30)

Fungicide*	Yeast isolate		
	LS11	LS28	LS30
Benlate (benomyl, 50)	<10	>500	>500
Siatek (tiabendazole, 41.8)	<10	>500	>500
Azuram (copper oxychloride, 40)	>100 < 500	>500	>500
Topas 10EC (penconazole, 10.2)	>10 < 20	>10 < 20	>10 < 20
Folicur (tebuconazole, 25)	>10 < 20	<10	<10
Sumisclex (procymidone, 50)	>500	>500	>500
Ronilan (vinclozolin, 41.8)	>500	>500	>500

*For each trade-product, the a.i. and its percentage content (w/w) are given in brackets.

1996 and 1997. The cv. Annurca of apple was used because of its high incidence of fungal rots. Apples received different treatments with fungicides and insecticides but application of these products was stopped 30 days before trials began. Soon after harvesting (October 19, 1996 and October 2, 1997) apples were placed in a single layer of sawdust on the ground in an orchard area, named 'melaio', and covered with a plastic shadowing net at 2 m above the ground in order to reduce direct sun radiation. According to the local farm schedule, apples were kept under these conditions for at least 20 days during which time they were periodically humidified to avoid drying and turned upside down to favour the peel reddening by sunlight.

Soon after harvesting, the antagonists (10^7 cfu ml^{-1}), suspended in water or in a solution of Benlate at a low dosage (10 g hl^{-1} of a.i. (benomyl)), were sprayed on fruits placed in the 'melaio' by a motorized sprayer (0.1 l of water delivered per m^2). After 30 min fruits were turned and treated again. Apples untreated or treated with fungicide at low (10 g hl^{-1} a.i.) or at full dosage (100 g hl^{-1} a.i.) were also included as controls. Plots with 150 apples, spaced 1 m apart and arranged in a complete randomized block design with 3 replications, were utilized in each treatment. In order to prevent spray drift apples of contiguous plots were covered by a layer of polyethylene during treatments.

When the apple skins were completely reddened (20 and 24 days after treatments, during 1996 and 1997, respectively), apples were taken from the 'melaio' and treated again for 30 s by dipping them in tanks containing yeast cell suspensions alone or plus the low dosage of benomyl (BLD). Fruits were arranged in commercial plastic trays and, after drying at room temperature, they were stored in a cold room at 3 °C with 90–95% RH for 60 days. At the end of cold storage fruits were kept for a shelf-life period of 15 days at 20 °C.

The severity of disease due to different fungal pathogens was assessed using the following empirical scale: 0 = fruit without decay; 1 = 1–20% of decayed fruit surface; 2 = 21–40% of decayed fruit surface; 3 = 41–60% of decayed fruit surface; 4 = 61–80% of decayed fruit surface; 5 = 81–100% of decayed fruit surface.

Microbial epiphytic populations

For each treatment, the microbial population was assessed on the surface of apples randomly sampled at the following times: (a) soon after the first treatment in 'melaio'; (b) at the end of apples conservation in 'melaio'; (c) soon after the pre-storage treatment of apples; (d) at the end of cold storage. In 1997, two additional samples were taken: halfway through apples conservation in 'melaio' and halfway through cold storage. Unwounded tissue samples were explanted from fruits with a cork borer (13 mm internal diameter). Ten explants of tissue taken from 3 fruits were suspended in 50 ml of sterile distilled water, shaken at 200 rpm for 30 min and after serial dilutions poured in Petri dishes containing BYA plus antibiotics (Lima et al., 1998a). For each treatment the samples were replicated three times. After incubation at 24 °C for 4 days, the colonies of four morphological groups of microorganisms (white yeasts, pink yeasts, yeast-like fungi and filamentous fungi) were recorded and expressed as colony forming units (cfu) per cm^2 of apple surface.

Identification of antagonists isolates by AFLP

During the studies aimed at monitoring yeast populations by agar plate method (see above), colonies of pink yeasts, white yeasts and yeast-like fungi were randomly selected from Petri dishes poured with washing water from apples treated or untreated with the biocontrol yeasts in semi-commercial conditions. Samples were taken in 1997 at the following times: (a) soon after the first treatment in 'melaio'; (b) at the end of apples

conservation in 'melaio'; (c) at the end of cold storage. For each treatment and sampling 15 yeast colonies were taken. Yeast cells were suspended in 15% glycerol and stored at −80 °C in Eppendorf tubes. Before AFLP analyses, the stored isolates were poured on nutrient yeast-extract agar (NYDA) and grown at 23 °C for 36 h.

DNA from each isolate was extracted according to Hoffman and Winston (1987). AFLP analysis was performed according to a simplified method reported by McLauchlin et al. (2000). Briefly, total genomic DNA was digested with *Hind III* (Gibco Life Technologies, Paisley, UK); the oligonucleotides adapters ADH1 and ADH2 (Primm, Milan, Italy) were ligated (DNA ligase, Gibco Life Technologies, Paisley, UK) to the mixture of restriction fragments. PCR amplifications were performed in a Perkin Elmer DNA Thermal Cycler 480 and PCR products were separated on 1.5% agarose gel.

Statistical analysis

Data of antagonistic activity assessed in small-scale experiments were submitted to variance analysis (ANOVA 1). The severity of disease, evaluated from apples in semi-commercial conditions by using the empiric scale described above, was used to calculate McKinney's index (McKinney, 1923) and submitted to variance analysis (ANOVA 2). The average values of each experiment were compared by Duncan's multiple range test. The percentages were converted into Bliss angular values (arcsine $\sqrt{\%}$) before analysis.

Results

Small-scale experiments on wounded apples

All the antagonists reduced the percentage of rots in apple wounds inoculated with *B. cinerea* or *P. expansum* on fruits stored both at 3 and 20 °C (Figure 1). Isolate LS30 was the most effective antagonist determining a decay reduction ranging from 89.0% to 91.0%; isolate LS28 gave a decay reduction ranging between 86.1% and 91.4% except against *B. cinerea* on apples stored at 3 °C where decay reduction was 55.7%. Isolate LS11 displayed its highest activity on apples stored at 3 °C reducing decay due to *B. cinerea* and *P. expansum* by 63.1% and 72.2%, respectively. At 20 °C, isolate LS11 was less effective reducing *B. cinerea* and *P. expansum* infections by 24.5% and 41.5%, respectively.

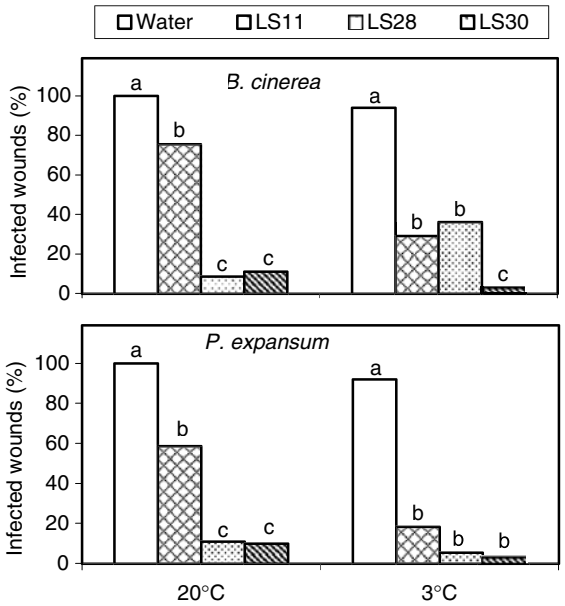

Figure 1. Antagonistic activity against *B. cinerea* and *P. expansum* of the biocontrol yeasts *R. glutinis* (LS11), *C. laurentii* (LS28) and *A. pullulans* (LS30) on apples. Wounded fruits were treated with a cell suspension of the antagonist or water (control) and after 2 h they were inoculated with a spore suspension of the pathogen. The percentage of infected wounds were assessed after 5 days of storage at 20 °C or after 30 days at 3 °C. For each pathogen, values marked by the same letters are not statistically different at $P = 0.01$, according to Duncan's multiple range test.

Sensitivity of antagonists to fungicides

Sensitivity of the antagonists towards some common fungicides *in vitro* is shown in Table 1. Isolates LS28 and LS30 were resistant to benzimidazoles (benomyl and thiabendazole), dicarboximides (procymidone and vinclozolin) and copper oxychloride at the highest tested concentration (500 mg l^{-1}). Also isolate LS11 showed a good resistance to dicarboximides (MIC > 500 mg l^{-1}) and copper (MIC > 100 < 500 mg l^{-1}), while it was appreciably inhibited by benzimidazoles (MIC < 10 mg l^{-1}). The 3 antagonists were sensitive to triazoles (penconazole and tebuconazole), with MIC values ranging from 10 to 20 mg l^{-1} and in some cases lower than 10 mg l^{-1}.

Semi-commercial apple treatments

Data regarding decays assessed on apples treated in semi-commercial conditions are shown in Figure 2.

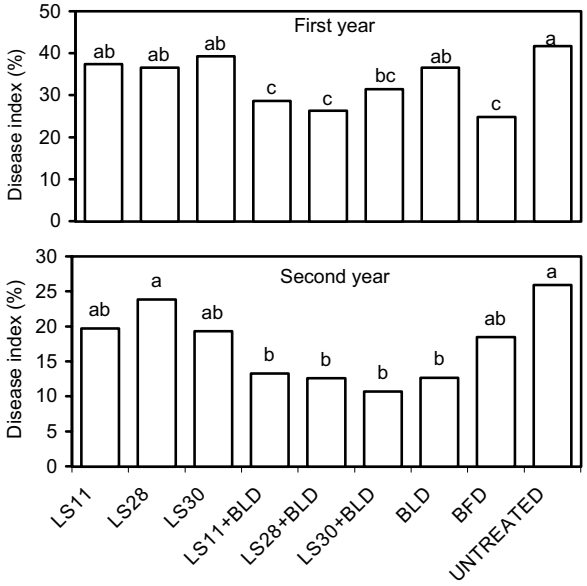

Figure 2. Fungal decays on apples treated twice in semi-commercial conditions with the biocontrol yeasts *R. glutinis* (LS11), *C. laurentii* (LS28) and *A. pullulans* (LS30) alone or combined with a BLD (10 g hl^{-1} a.i.). Apples were stored at 3 °C for 60 days with 95–98% RH, plus 15 days of shelf life at 20 °C. The trials were performed in 1996 (first year) and 1997 (second year). For each year, values marked by the same letters are not statistically different at $P = 0.01$, according to Duncan's multiple range test. BFD = benomyl at full dosage (100 g hl^{-1} a.i.).

The severity of rots on untreated apples at the end of cold storage was 42% in 1996 and 26% in 1997. The main fungal pathogens found on naturally-infected apples were *P. expansum*, *B. cinerea* and *Monilinia* spp.

In both years, all the antagonists applied in combination with a BLD reduced fruit rots compared to the untreated controls. The integrated control resulted in an activity comparable to that exerted by benomyl applied at full commercial dosage. Only slight reductions of decays were obtained when the antagonists were applied alone.

In comparison to untreated controls, isolates LS11, LS28 and LS30 applied with benomyl reduced the rot incidence by 31.4%, 36.9% and 24.7% in the first year and by 49.0%, 51.3% and 58.8% in the second year, respectively. The fungicide at low dosage applied alone reduced the incidence of decay by 12.2% in 1996 and by 51.2% in 1997. The full dosage of fungicide reduced rot incidence by 40.5% in the first year and only by 28.9 in the second year.

Microbial epiphytic population

Populations of yeasts on apples in semi-commercial conditions are reported in Figure 3. In both years, population levels of pink and white yeasts and yeast-like fungi on biocontrol yeast-treated apples were higher than those found on control apples in most of the cases, regardless of the presence of fungicide. The highest differences among levels of populations on yeast-treated apples and control apples (populations averaged from 100- to 1000-fold higher) were observed during the cold storage period and particularly in 1997.

In both years, following the first treatment in the orchard, the population of each morphological yeast group on apple skins treated with biocontrol yeasts generally reached values of about 10^5 cfu per cm^2. During the period of fruit conservation in 'melaio', yeast populations on apples treated with antagonists alone generally increased in the first year, while they declined appreciably in the second year. In any case, values were most frequently in the range from 10^3 to 10^4 cfu per cm^2; these trends were not appreciably influenced by the presence of benomyl at low dosage (Figure 3). In both years, the dip treatment preceding cold storage, determined with some exceptions, a general reduction of yeast populations on apples. During cold storage periods populations of all yeast groups on apples remain substantially unchanged, except for white yeasts, which decreased in the second year.

On control apples the fungicide at full dosage produced some decreases of the yeast populations. The treatments with antagonists or antagonists plus benomyl generally reduced population of filamentous fungi just as the fungicide applied alone at full dosage did (data not shown).

Identification of antagonist isolates by AFLP

In preliminary experiments, AFLP fingerprints of isolates LS11, LS28 and LS30 were compared with those of at least 20 yeast isolates chosen among those obtained from surfaces of apple and other crops and belonging to the same genera and/or species of microorganisms. In these experiments the molecular markers of the biocontrol yeasts resulted specific and reproducible (data not shown). In Figure 4, the AFLP patterns of the pure culture of the 3 biocontrol yeast are compared with those of the same strains after re-isolation from apples treated in semi-commercial conditions and also with patterns of the most representative strains of

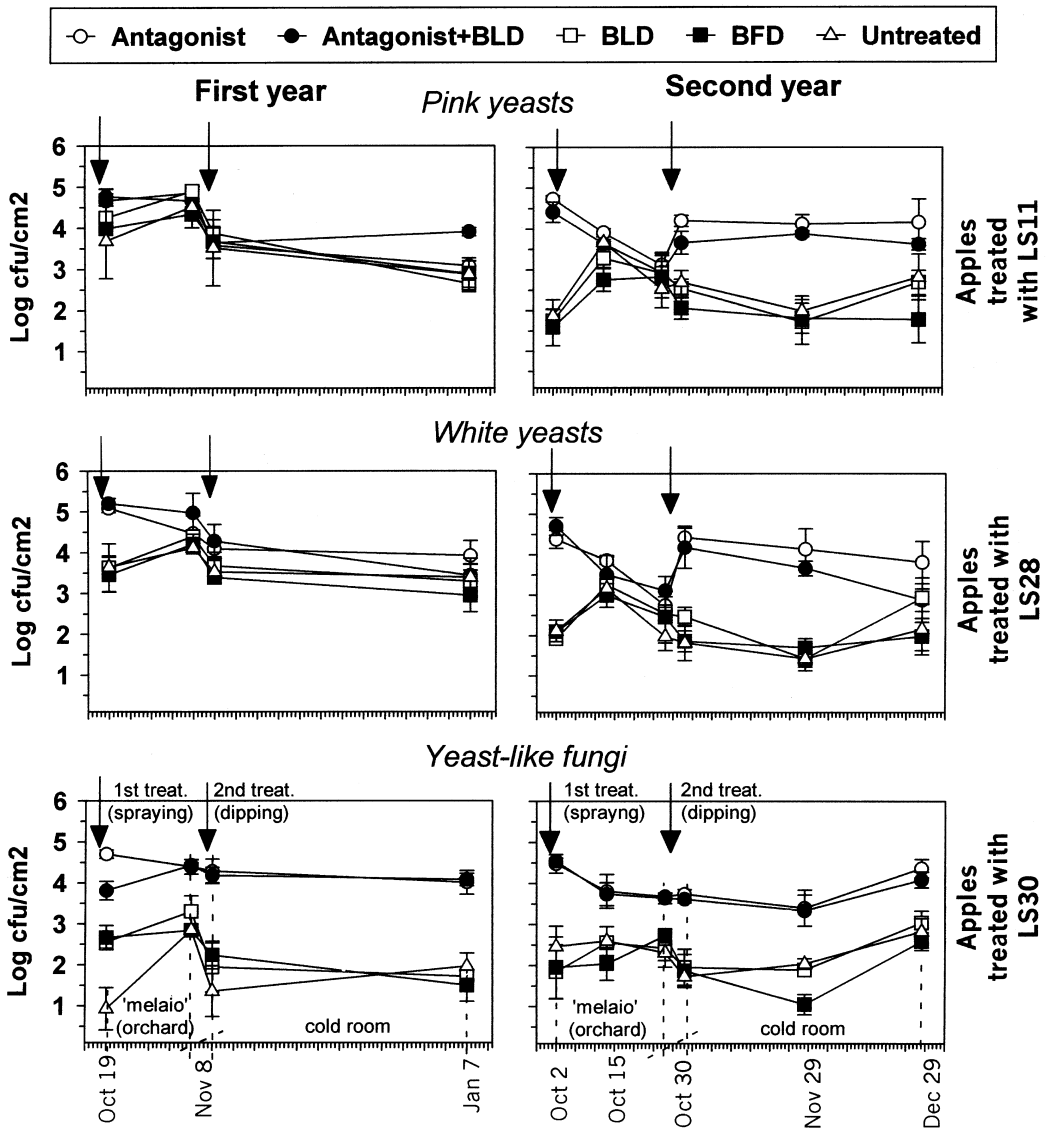

Figure 3. Populations of pink yeasts, white yeasts and yeast-like fungi on apple skin treated twice (arrows) with the biocontrol yeasts *R. glutinis* (LS11, pink yeast), *C. laurentii* (LS28, white yeast), *A. pullulans* (LS30, yeast-like fungus) alone or combined with a BLD. Apples were stored at 3 °C for 60 days with 95–98% RH. Bars on graphs represent the standard deviations from the means. On November 8 (first year) and October 30 (second year) populations were assessed twice (before and after dip treatment) in the same day. BFD = benomyl at full dosage.

indigenous yeasts found on untreated apples. The patterns of the yeast isolates recovered after application in apple surface (asterisks) corresponded to those of the original isolates applied (arrows), while they were different from patterns of indigenous yeasts found on untreated apples regardless of sampling time. In 95–100% of the analyses the patterns of re-isolated yeasts corresponded to those of the isolates applied.

Discussion

In small-scale experiments, isolates LS28 (*C. laurentii*) and LS30 (*A. pullulans*) showed a high level of antagonistic activity against *B. cinerea* and *P. expansum* on wounded apples stored both at 3 and 20 °C. Isolate LS11 (*R. glutinis*) was less active at 20 °C against both pathogens, while it showed a high activity (as high as

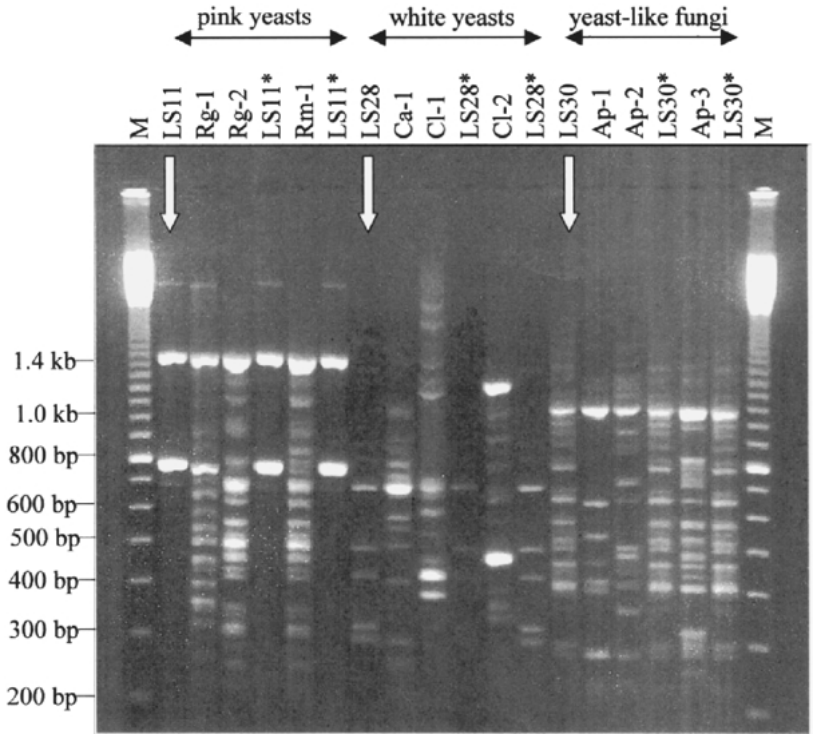

Figure 4. AFLP patterns of the biocontrol yeasts *R. glutinis* LS11, *C. laurentii* LS28 and *A. pullulans* LS30 from pure cultures (arrows) or after re-isolation from apple surfaces (asterisks), in comparison with the patterns of some indigenous yeasts (Rg = *Rhodotorula glutinis*; Rm = *R. mucillaginosa*; Ca = *Cryptococcus albidus*; Cl = *C. laurentii*; Ap = *Aureobasidium pullulans*) belonging to each morphological group and randomly isolated from apple surfaces.

isolate LS28) on cold stored apples (Figure 1). Accordingly, this isolate was effective in semi-commercial trials where apples were stored for 60 days in a cold room at 3 °C (Figure 2). The good activity of LS11 at low temperature could be partially explained by the higher unsaturation of fatty acids present in the polar lipid (membrane) fraction of LS11, which makes the membranes of this isolate more fluid and functional at low temperature (Castoria et al., unpublished).

The biocontrol yeasts, tested *in vitro* for resistance to low dosages of common fungicides, showed a good tolerance towards copper oxychloride and dicarboximides, while they were strongly inhibited by triazoles (Table 1). These results are in agreement with previous researches carried out with other yeast isolates (Lima et al., 1997). Triazoles, therefore, should not be utilized in combination with biocontrol yeasts in integrated control programmes. Isolates LS28 and LS30 were also resistant to benzimidazoles, while isolate LS11 was not.

In semi-commercial conditions, the 3 yeasts reduced fungal rots on apples when applied in combination with benomyl at low dosage determining decay reductions ranging from 25% to 59% (Figure 2). Such degrees of activity are considered economically consistent under commercial conditions (Chand-Goyal and Spotts, 1997). Yeasts plus fungicide at low dosage (only 10% of the commercially recommended dosage) showed an activity comparable to that of the fungicide applied alone at full dosage. In the first year, integration of yeast isolates LS11 and LS28 with benomyl showed also a clear synergistic effect. This is in agreement with results reported by other authors (Chand-Goyal and Spotts, 1997; Ippolito et al., 1998). In the second year, the integration of yeasts and benomyl, although reduced decay incidence with respect to untreated control, showed an activity similar to that of fungicide alone at low dosage, whose activity was even not different from that of the fungicide at full dosage. This result could be partially due to the lower incidence of natural decays (about 25% in the first year and about 40% in the second year in the control apples). However, the results should be considered as positive ones since the repeated application of benomyl, a fungicide

whose activity is based on a single and specific mode of action (its target is a single gene product), at low dosage alone, increases the risks for production of resistant pathogen strains. The combination of yeast isolates LS11, LS28 and LS30, acting by different and complex mechanisms of action (Castoria et al., 1997; 2001), with a reduced dosage of benomyl, exerts a good control of postharvest decay and reduces such risks. In this regard, Chand-Goyal and Spotts (1997) in the presence of 75% population of *P. expansum* resistant to benzimidazoles, observed good control of postharvest decays only on apples treated with a biocontrol yeast plus a reduced dosage of thiabendazole, while at full dosage the fungicide alone yielded unsatisfactory control. Finally, the application of the yeasts with a low dosage of fungicide in comparison with a treatment based on fungicide at full dosage alone clearly reduces risks for accumulation of residues in apples.

Isolate LS11, in spite of its sensitivity to benomyl (Table 1), was as effective as the fungicide-resistant isolates LS28 and LS30 (Figure 2) when combined with this fungicide in semi-commercial conditions. Duration and intensity of interaction between yeast and fungicides *in vivo* (on fruits) is presumably lower than *in vitro*, where the yeast remains in contact, for a longer time, with a dosage of fungicide substantially unchanged with respect to that initially applied. Benomyl is a systemic fungicide; after application on apples surface, part of it penetrates the fruit, while the biocontrol yeasts remain on the skin surface. Moreover, the residual amount of fungicide on fruit surface can gradually decrease due to degradation by environmental factors (Delp, 1987).

The antagonists applied alone in semi-commercial conditions showed slight activity with respect to the higher activity exerted in small-scale experiments on artificially wounded fruits. The more drastic conditions of semi-commercial trials, i.e., the possible presence of latent infections, could affect their biocontrol activity. However, it is worth mentioning that the antagonist cells were applied in water without adjuvants. Therefore, antagonist survival and activity might be enhanced by applying them as an appropriate formulation, e.g., combining the yeasts with some compatible substances (Lima et al., 1998b).

Data of population monitoring demonstrate that on fruit surfaces the cfu of antagonists reached values around 10^5 per cm^2 soon after application and some days later their populations generally decreased, remaining stable in the range from 10^3 to 10^4 cfu per cm^2 (Figure 3). This result, also observed by Leibinger et al. (1997), represents a good rate of survival for antagonists applied on plant surfaces. However, higher values of antagonist populations might be required to obtain a better control of decays. Fokkema et al. (1979) suggested that at least 10^4 cells per cm^2 of yeast isolates are necessary to control necrotrophic fungal pathogens on rye and wheat leaves.

The morphologically-based monitoring of biocontrol yeasts was integrated using specific fingerprints obtained by AFLP. This technique, developed by Vos et al. (1995) was used for genetic characterization and fingerprinting of different organisms due to its capacity to detect high levels of polymorphism. In our study, we used a simplified AFLP protocol that gives reproducible and reliable fingerprints and is also easier to perform (McLauchlin et al., 2000). The AFLP fingerprints of isolates LS11, LS28 and LS30 were applied with success to confirm their identity after reisolation from apples treated in semi-commercial conditions (Figure 4) and to improve their monitoring. The availability of molecular fingerprints is an important pre-requisite requested by potential companies interested in the development of commercial formulations based on biocontrol agents, since it prevents their unauthorized use. Using the AFLP technique, researches are in progress to develop isolate-specific primers or probes to further improve identification and monitoring of our biocontrol yeasts.

In conclusion, this study demonstrates that utilization of integrated control, combining yeast isolates with low dosage of fungicides, could be an effective strategy in reducing postharvest fungal decay and its related risks. Further investigations are in progress to optimize activity, application and monitoring of the biocontrol agents.

Acknowledgement

The authors gratefully acknowledge Dr D.F. Cezza for linguistic advice.

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